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Simple and rapid methods for the analysis of Sulfonamide bacteriostatic antibiotic in dosage forms

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ABSTRACT

Two novel simple, precise and rapid spectrophotometric methods (Mehod A and Mehod B) are proposed for the determination of sulfamethaxazole (SFZ) using ferric chloride and potassium ferricyaide, sodium nitrite and resorcinol, as reagents. Method A is based reduction of Fe^{+3} by SFZ and Fe^{+2} produced forms complex with potassium ferricyanide to form a green colored chromogen having maximum absorption at 720nm. Method B is based on the diazotization of the drug by sodium nitrite in acidic medium at 5°C followed by coupling with resorcinol to form yellow colored species (λ_{max} 430nm). Beer's law is obeyed in the ranges, 4-20 and 2-10 µg/mL for method A and method B, respectively. The apparent molar absorptivities are calculated to be 0.61 x 10^4 and 1.089 × 10^4 L $mol^{-1}cm^{-1}$ for method A and method B, respectively, and the corresponding Sandell sensitivity values are 0.04 and 0.023 ìg cm^{-2}. The methods were successfully applied to the assay of SFZ in pharmaceutical formulations with satisfactory results.

Key words: Sulfamethaxazole (SFZ), Sandell's sensitivity, Visible Spectrophotometric determination, Beer's Law.

INTRODUCTION

Sulfamethaxazole is a sulfonamide bacteriostatic antibiotic. Sulfonamides are structural analogs and competitive antagonists of para-aminobenzoic acid (PABA). They inhibit normal bacterial utilization of PABA for the synthesis of folic acid, an important metabolite in DNA synthesis[1,2]. These are used in the treatment of urinary track infections, eye infections and as a prophylaxis of rheumatic fever[3]. The drug has been determined by a variety of analytical techniques such as high performance liquid chromatography[4,5], high performance thin layer chromatography[6], gas chromatography[7], electro analytical methods[8-11], spectrophotometry[12-16] and differential scanning calorimetry[17]. By exploiting the various functions groups in the SFZ the authors had developed two simple and sensitive spectrophotometric methods for the determination of SFZ in pharmaceutical formulations and biological samples.

EXPERIMENTAL

Apparatus

Systronics UV – Visible Double beam spectrophotometer model 2201.

Materials and Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared in distilled water.

Method A

- 0.25% Potassium ferricyanide: Prepared by dissolving 250mg of $K_4Fe(CN)_6$ in 100ml of distilled water
- 0.5 % $FeCl_3$: Prepared by dissolving 500mg of ferric chloride in 100ml of distilled water.

Method B

- 0.1% Sodium nitrite: Prepared by dissolving 100mg of sodium nitrite in 100mL of distilled water.

- 5N HCl
- 0.25% Resorcinol: Prepared by dissolving 250mg of resorcinol in 100mL of distilled water

Preparation of standard and sample solution

Accurately weighed 100mg of SFZ was dissolved in 2mL of 10M H_2SO_4 and made up to 100mL with distilled water to give a concentration of 1mg/mL. The final concentration was brought to 200 µg/mL for both Methods A and B.

General procedure for the determination of Sulfamethaxazole

Method A

Aliquots of the working standard solution of SFZ (200µg/mL) were transferred into a series of 10 mL calibrated flasks. To all the flasks 1mL of potassium ferricyanide and 1mL of 0.5% $Fecl_3$ were added. The solution was made up to the mark with distilled water, mixed well and kept aside for 10 min. The absorbance of the green colored chromogen was measured at 720 nm against the corresponding reagent blank.

Method B

Aliquots of working standard solution of SFZ solutions (200µg/mL) were transferred into 10-mL calibrated flasks followed by 2.0 mL 5N HCl to each. After cooling in an ice bath, 2 mL of 1% sodium nitrite was added under swirling. The solutions were allowed to stand for 5 min and then 1 mL of 0.2% resorcinol was added. The solution was made up to the mark with distilled water, mixed thoroughly and after 5 min the absorbance was measured at 430 nm against a reagent blank, and the calibration graph was constructed.

Assay of pharmaceutical tablets

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 100 mg of the SFZ was dissolved in water and filtered. The filtrate was made up to 100 mL and appropriate aliquots of the drug solution were treated as described above for the determination of SFZ.

RESULTS AND DISCUSSION

Spectral characteristics

Method A involves the reduction of the Fe^{+3} by the SFZ and Fe^{+2} produced forms complex with

Table 1: Optical and regression characteristics, precision and accuracy of the proposed methods

Parameters	Method A	Method B
λ_{max} (nm)	720	430
Beer's law limit (µg/ mL)	4 – 20	2 - 10
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.04	0.023
Molar absorptivity(Litre.mole^{-1}.cm^{-1})	0.61 x 10^4	1.089 x 10^4
Stability of Color (hours)	24	48
Regression equation (Y)*		
Intercept (a)	0.001	0.0038
Slope(b)	0.022	0.0040
% RSD$	0.92	1.04
% Range of errors (95% confidence limits):		
0.05 significance level	0.769	0.869
0.01 significance level	0.138	1.286
Correlation coefficient	0.999	0.999

* Y= a + bx, where Y is the absorbance and x is the concentration of Sulfamethaxazole in µg/ mL

$ = for six replicates

potassium ferricyanide to form a green colored chromogen having λ_{max} 720nm. Method B involves the diazotization of SFZ, followed by coupling with resorcinol. The absorption spectra of $Fe_4[Fe(CN)_6]_3$ complex and SFZ-resorcinol complex have absorption maxima at 720 and 430 nm respectively.

Analytical data

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from six replicate samples) were calculated for all the methods and the results are summarized in Table 1. Regression characteristics like standard deviation of slope (b), standard deviation of intercept (a) and % range of error (0.05 and 0.01 confidence limits) and were calculated for all the methods and are shown in Table 1.

Table 2: Results of analysis of tablet formulations containing SFZ

Formulations	Labeled amount(mg)	Recovery by reference method*(%)	Recovery by proposed methods (%)**	
			Method A	Method B
Tablet I	100	99.90	99.95	99.96
Tablet II	100	99.86	99.70	98.60
Tablet III	100	98.76	99.80	99.69

* Reference method was UV method developed in the laboratory.

** Recovery amount was the average of six determinants.

Analysis of pharmaceutical preparations

Application of the proposed methods to the determination of SFZ in its dosage forms was successfully made; the results are presented in Table 2. The excellent recoveries obtained indicated the absence of any interference from the excipients.

CONCLUSION

The proposed methods were found to be simple, economical, selective and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing SFZ showed no interference from the common excipients. Hence, these methods could be considered for the determination of SFZ in the quality control laboratories.

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